Changes of delayed luminescence spectra in rice of different polluted degree by *Aspergillus flavus*

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Light-induced delayed luminescence (DL) of living organisms contains information on the quality of the living state of these organisms. Employing a LS-55 Luminescence Spectrometer, the changes of DL spectra of rice with *Aspergillus flavus* treating different time were observed. Rice with *Aspergillus flavus* treatment for a shorter time had a stronger intensity of DL. The polluted degree of *Aspergillus* showed a negative correlation with the intensity of DL. Comparing with the intensity of DL, we found that the concentration of aflatoxin in different polluted degree rice had negative correlation with the intensity of DL. We believe DL technique may be helpful in elaborating a fast, holistic, and non-invasive method for the rapid evaluation the polluted degree of rice by *Aspergillus flavus*.

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All organisms emit weak light spontaneously at very low rates, which is called luminescence. When illuminated with a pulse of light, a much higher rate of re-emission occurs and decays hyperbolically within seconds to minutes. This phenomenon is called delayed luminescence (DL) and its lifetime extends from 10^{-7} to more than 10 s. In the past, it has been found that there was a close connection between the state of living organisms and their DL in many biological systems[1,2,3].

Aflatoxins are a group of toxic secondary metabolites of fungi, most commonly produced by certain strains of *Aspergillus flavus* and all strains of *Aspergillus parasiticus*. A variety of crops including peanuts, corn, cotton seed, rice, wheat, copra, peas, millet, chillies, spices, and dry fruits may be contaminated with aflatoxins, particulary aflatoxin B1 (AfB1). Amongst animals, poultry livestock are particularly endangered by acute aflatoxicosis. Prolonged exposure to subacute levels of aflatoxins is detrimental to human health. The contamination of agricultural commodities with aflatoxin is established concern at both national and international levels. The regulations governing aflatoxin levels in food and feed are globally and stringent, with enforcement of regulatory limits. To measure AfB1 at the concentrations present in food commodities, several chromatographic methods[4−7] have been used liquid chromatography (LC) with fluorescence detection (FLD)[8] or mass spectrometry detection (MS)[9] are the most employed, Niedwetzki *et al.*[10] developed an automatic work station for determination of aflatoxins based on LC.

However to date, DL has not been used for analysis of aflatoxins from rice. This report presents novel DL spectra methods for determination of AfB1 in intact rices with *Aspergillus flavus* treatment for different time, comparing with the common fluorescence detected method as a tool for routine analysis of aflatoxins in rice. The detection of AfB1 concentrations in different polluted degree rice with delayed fluorescence spectrum methods has not previously been reported.

Polished rice was bought from Guangzhou market. AfB1 were obtained from the Sigma Chemical Co. *Aspergillus flavus* A s3. 2890 (supplied by Crops Research Institute, Guang dong Academy of Agricultural Sciences) was used to inoculate rice.

Choosing 10 gram health rice, sterilizing 1 min with 70% medicine ethanol, washing-up 3 times with 5 ml of water were sterilized in autoclave (121 °C, 20 min) and then it was inoculated with 1 ml of a homogeneous suspension (107 conidia/ml) of *Aspergillus flavus*, shaking up with sterilized scoop and then push out 1 ml of a homogeneous suspension, incubated in sterilized glass vessel for 0, 12 h, 18 h, 24 h, and 41 h at in an artificial climate box (LRH-250-GS) at 25 °C in the darkness. Aflatoxins extract analysis was performed as described previously[11,12].

DL spectra were measured using a LS-55 luminescence spectrometer (manufactured by Perkin Elmer, USA) with excitation wavelength 365 nm, excitation slit of 2.5 nm, emission slit of 2.5 nm, and operating in phosphorescence mode, scan speed of 200 nm/min. The excitation wavelength of 365 nm was chosen for fluorescence spectra generation because 365 nm is known to excite AfB1.

Fluorescence spectra were measured using a LS-55 luminescence spectrometer (manufactured by Perkin Elmer, USA) with excitation wavelength of 365 nm, excitation slit of 2.5 nm, emission slit of 2.5 nm, and operating in fluorescent mode, scan speed of 200 nm/min.

Figure 1 showed the rice appearance and color after *Aspergillus flavus* treating different time, 1, 2, 3, 4, and 5 stand for treating 0, 12 h, 18 h, 24 h, and 41 h with

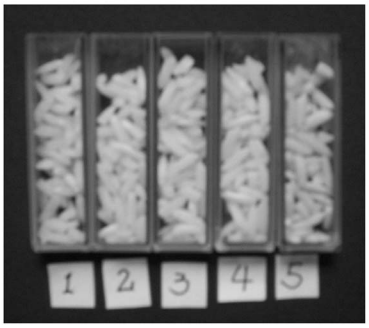

Fig. 1. Imaging of rices in different polluted degree of *Aspergillus*. 1, 2, 3, 4, and 5 stand for 0, 12 h, 18 h, 24 h, and 41h after treating with *Aspergillus*.

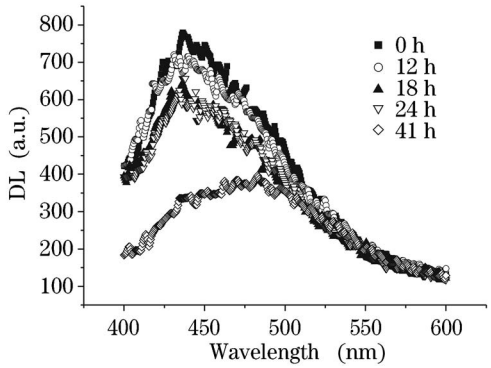

Fig. 2. DL emission spectra of rices at 0, 12 h, 18 h, 24 h, and 41 h after treating *Aspergillus* (excitation at 365 nm).

Aspergillus flavus. DL spectra were showed in Fig. 2, the whole rice with *Aspergillus flavus* treatment for a longer time had a thinner intensity of DL (excitation light 365 nm). The polluted degree of *Aspergillus* showed a negative correlation with the intensity of DL.

Fluorescence emission spectra of whole rice in different polluted with *Aspergillus flavus* were showed in Fig. 3. The results showed that the fluorescence emission spectra of whole rice would be similar comparing with the DL spectra. The emission peak is also 440 nm, the intensity of fluorescence at 440 nm showed a negative correlation with the polluted degree of *Aspergillus flavus*.

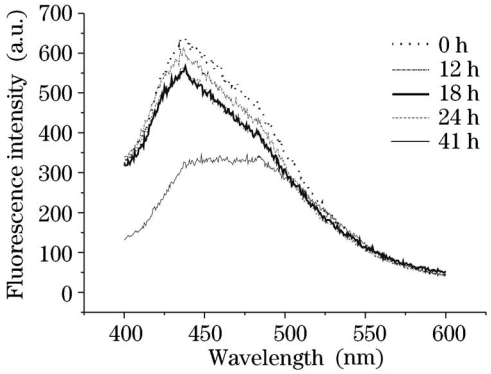

Fig. 3. Fluorescence emission spectra of rices at 0, 12 h, 18 h, 24 h, and 41 h after treating *Aspergillus* (excitation at 365 nm).

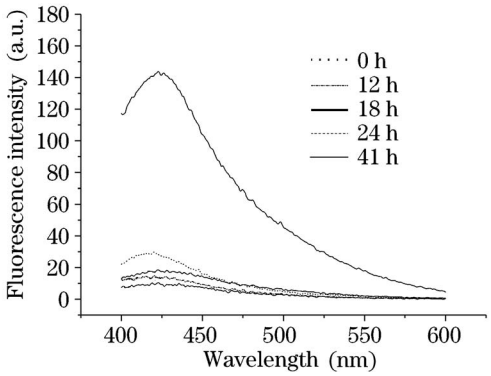

Fig. 4. Fluorescence emission spectra of methanol–water extraction rice at 0, 12 h, 18 h, 24 h, and 41 h after treating *Aspergillus* (excitation at 365 nm).

In the last years many biological systems have shown this close connection between the biological state of the system and physical parameters of DL. It is known that *Aspergillus flavus* can produce aflatoxin. With fluorescence spectra, the aflatoxin concentration of *Aspergillus flavus* treating rice different time were measured.

Fluorescence detector is a common method used for determination of aflatoxins in contaminated food[13]. The common solvent used for aflatoxins extraction is mixtures of methanol-water[12]. Aflatoxins were extracted with 10 ml of a mixture of methanol-water (55:45, v/v) with magnetic stirring for 30 min. The extract was filtered through Whatman filter paper. Detection of aflatoxins was carried out using 365 and 435 nm as wavelengths for excitation and emission, respectively. The results were showed in Fig. 4. Comparing with the concentration of aflatoxin in different polluted degree rice, the intensity of DL (Fig. 5) and fluorescence (Fig. 6) at peak values had negative correlation.

The determination of aflatoxinB1 is difficult due to the need to detect them in complex mixtures and at low concentrations. Many of the analytical methods currently published for the determination of aflatoxins employ solvent extraction with harmful solvents or immunoaffinity columns and multifunctional columns that are very expensive and matrix dependant[14−16]. We found that DL spectra method directly measured with whole rice might be applicable to the determination of AfB1 concentration. The DL spectra methods are potential ways in elaborating a fast, quantitative and no harmful method for the rapid determination of AfB1 in rice.

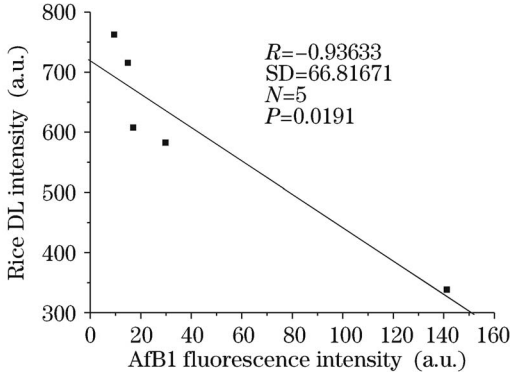

Fig. 5. Relationship between AfB1 in rice and the intensity of whole rice DL spectra at peak.

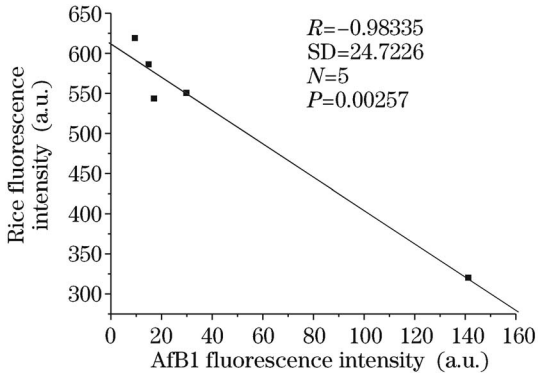

Fig. 6. Relationship between AfB1 in rice and the intensity of whole rice fluorescence spectra at peak.

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